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REVIEW

Recent Studies of Fish Pancreatic Hormones: Selected Topics

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INTRODUCTION

Interest in fish pancreatic hormones has grown substantially during recent years. This interest stems not only from the fact that Brockmann bodies of fish provide a useful model for the processing and secretion of peptide hormones, but also from the conviction among biologists that fish research promises both theoretical and practical benefits [1].

Various aspects of progress in the field of fish pancreatic hormones and their roles in regulation of metabolism in cyclostomes and fishes have been reviewed recently [2–7]. Epple and Brinn's [8] book "The Comparative Physiology of the Pancreatic Islets", an encyclopedic source of information, deals with most aspects of the structure of the vertebrate pancreas and the functions of its active peptides. Specific details of the amino acid sequences of pancreatic hormones and their biosynthetic pathways in fish, as compared to other vertebrates, have been thoroughly covered by Conlon [9]. Nevertheless, new information in this field is accumulating so rapidly that it seems worthwhile at this time to summarize the latest trends and findings in the studies of the fish endocrine pancreas. Most references to the actions of mammalian pancreatic hormones on fish were deliberately omitted because these topics have been repeatedly and adequately discussed in the literature [3, 8, 10].

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List of abbreviations used

aPY or YG-anglerfish pancreatic peptide Y
CCK-cholecystokinin
EGF-epidermal growth factor
ELISA-enzyme linked immunoassay for soluble antigens
GLP-glucagon-like peptide
GLU-glucagon
GH-growth hormone
IGF-1, IGF-2-insulin-like growth factors 1 and 2
INS-insulin
NPY-neuropeptide Y
PP-pancreatic polypeptide
RIA-radioimmunoassay
sPP-salmon pancreatic polypeptide
SST-somatostatin
YY-peptide YY

IMMUNOCYTOCHEMICAL AND STRUCTURAL STUDIES OF PANCREATIC HORMONES

Reports of basic immunocytochemical investigations, as well as correlative immunocytochemical and electron microscopical studies, continue to be numerous [11–20]. A typical pattern of recent research of this type is the use of several mono- or polyclonal antisera (instead of one), raised against the same antigen, but recognizing different oligopeptide or polypeptide fragments of hormone molecule.

Employing this approach McDonald *et al.* [21], discovered that two peptides of the somatostatin (SST) family, SST-14 and SST-28, which in the anglerfish (*Lophius americanus*) are the products of two separate genes (gene I and gene II), are expressed in different types of pancreatic cells. Moreover, the islet cells that process the product of gene II, (so called SST-28-II) were localized in close association with glucagon-immunopositive (GLU-immunopositive) cells. This observation was extended by Nozaki *et al.* [17] who found that, in salmon and trout Brockmann bodies, the cells producing SST-25-II were in close topographical association with GLU-immunopositive cells, while the cells producing SST-14-I, were located more centrally, in association with insulin-immunopositive (INS-immunopositive) cells (Fig. 1). This finding suggests that, in teleostean fish, such as catfish (*Ictalurus* sp.), eel (*Anguilla anguilla*), sculpin (*Cottus scorpius*) and probably many others, which, in contrast to mammals, possess two separate sets of genes for SSTs [3, 4, 9, 22], these SSTs will also be found in different types of cells. Indeed, Abad *et al.* [13] reported recently that, in the Brockmann body of gilthead sea bream (*Sparus auratus*), from which the gene II SST has not been yet isolated, immunostaining with antibodies against SST-25-II from salmon and against mammalian SST-14-I, follows a pattern similar to that in the anglerfish, salmon and trout. These observations lead, naturally, to several questions that need to be addressed. First, why is there such a specific separation of anatomical sites of production of the two SSTs, since some other peptides, even if they belong to different families, such as GLU and pancreatic polypeptide (PP), often coexist in the same cells and, moreover, in the same secretory granules [reviewed in 12, 18]? Second, does the close topographical association between cells that produce SST-25-II with GLU cells and between cells that produce SST-14-I with INS cells, imply an as yet unknown functional relationship between these pancreatic peptides in fish? Yet another enigma to be resolved is the distribution in the brain of some "big" SSTs, which are either the products of gene II (e.g., in salmonids, catfish, sculpin, anglerfish and eel), or truncated products of gene I [lamprey, 23; hagfish, 24]. Morel *et al.* [25], came to the conclusion that processing of two distinct precursors of SSTs in the teleostean fish operates "in a fixed pattern rather than in a tissue-specific manner", however, they found that in the pancreas of anglerfish the product of gene II (SST-28-II) prevails while in the brain the level of SST-28-II is very low. In the gut cells only traces of SST-28-II could be detected. Nozaki *et al.* [17] failed to find any cells that were immunopositive for SST-25-II in the neurohypophysis or hypothalamus of either Pacific salmon or trout, while cells positive for SST-14-I were abundant. It is noteworthy that Marchant *et al.* [26] and Marchant and Peter [27] found that neither catfish SST-22-II, nor salmon SST-25-II inhibited the release of growth hormone (GH), while SST-14-I retained its full inhibitory potency. Therefore, the abbreviation SRIF (somatotropin release inhibiting factor) does not seem to be applicable to the SSTs encoded by the gene II family. SST-14-I was the only SST that has been found in the pancreas and in the gut of cartilaginous fishes [28]. In these fishes, the distribution of SST and GLU-immunoreactivities suggests a possible regulatory role of both peptides in gastric secretion and/or cell proliferation. Moreover, paracrine interrelationships between GLU and SST-14-I have been suggested both in the pancreas and in the gut [29, 30].

In dogs, only SST-28-I (the amino-terminal extension of SST-14-I) from the stomach and intestine seems to respond to physiological stimuli. The pancreatic SST-14-I, by contrast, is believed to have mainly a nonhormonal, local paracrine function [31–35]. What then is the situation in lam-

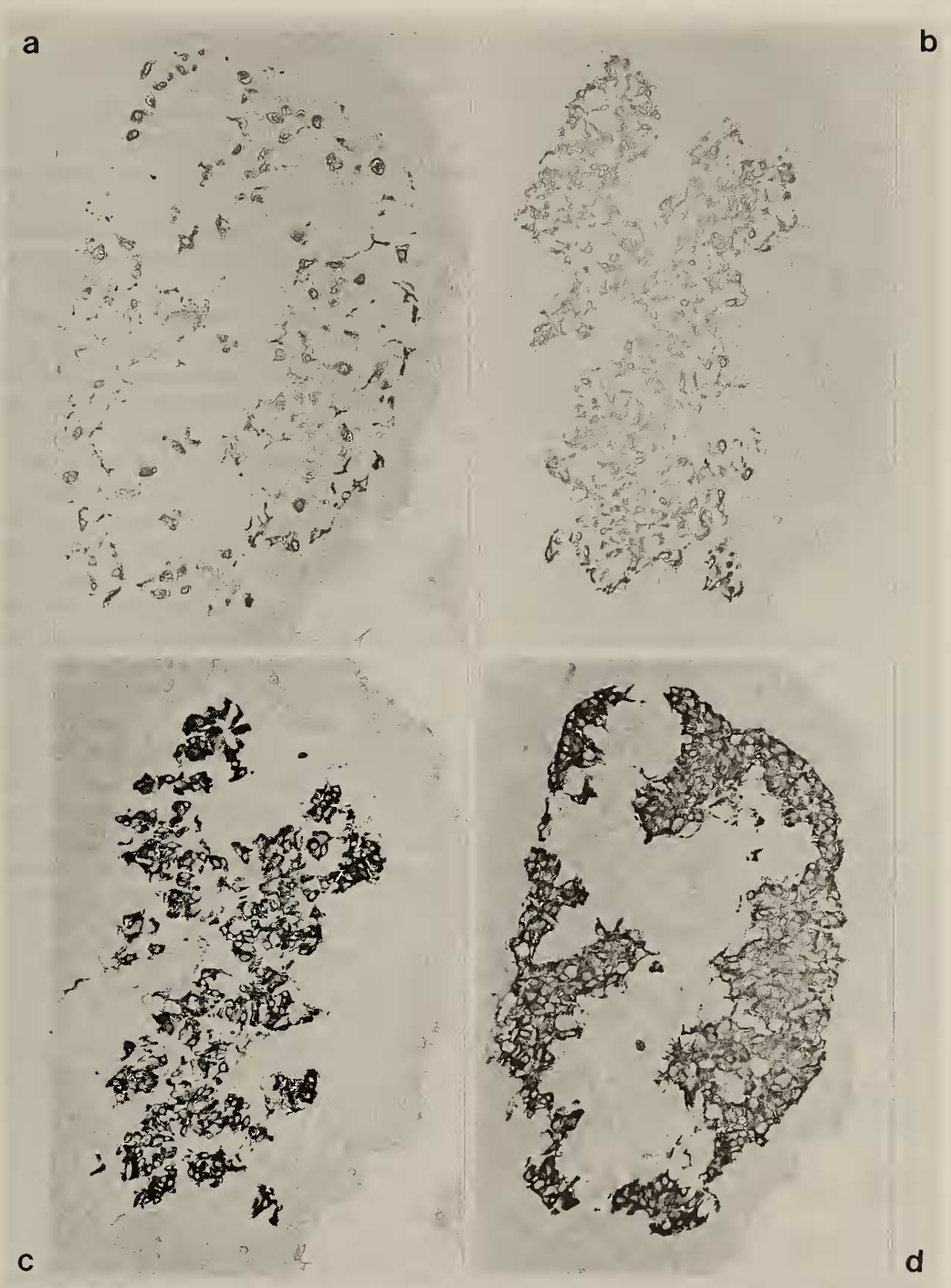

FIG. 1. Four successive sections of a rainbow trout pancreas stained differentially with antibodies against salmon SST-25-II, preabsorbed with SST-14-I (a); antibodies against mammalian-type SST-14-I (b); antibodies against salmon insulin (c) and antibodies against salmon glucagon (d). Note the topographical differences in the distribution of cells that contain SST-25-II-like (a) and SST-14-I-like (b) immunoreactivities. Note also topographical associations between the cells containing sSST-25-I-like and glucagon-like immunoreactivities (a, d) and SST-14-I-like and insulin-like immunoreactivites (b, c) respectively. From reference [17] (Gen. Comp. Endocrinol., with permission).

preys and teleost fish, in which the pancreatic cells that express gene I SST-34 (lamprey) or one of gene II SSTs (teleosts) are either present in equal numbers or are more abundant than any other types of cells, and "big" SSTs are the major peptides processed in the islet organ [16, 17, 23, 36, 37]? Is the role of these pancreatic SSTs confined to paracrine effects on adjacent GLU/GLP cells (in teleost fish) or upon INS-secreting cells (in lampreys)? Do these SSTs also have metabolic or still other potencies, as has been reported by Sheridan *et al.* [38]? Is there any functional difference between SSTs of the gut and those of pancreatic origin in lampreys, which have abundant SST-immunopositive cells located both in the gut and in the pancreatic islets [16, 36]?

In 1988 much progress was made in the elucidation of the primary structures of pancreatic hormones of agnathans (hagfish and lamprey), the only two current representatives of the most primitive vertebrates. Pancreatic SSTs of hagfish (*Myxine glutinosa*) and lamprey (*Petromyzon marinus*) were isolated and their amino acids sequenced [23, 24]. SSTs of lamprey are peptides of 34-37 amino acids with SST-34-I, as the predominant form. Hagfish SST is also a peptide of 34 amino acids and it is strikingly similar to lamprey SST at the carboxyl end, where 17 of the amino acids are identical. By contrast, 16 other amino acids in the hagfish and lamprey SST-34 are completely different, and only 2 of them are in identical positions [23] (Fig. 2). Agnathan SSTs are the result of significant differences in the processing of proSSTs (precursors to SSTs) as compared to the processing of SSTs in either teleost fish or mammals: in both hagfish and lamprey, a series of amino-terminally truncated peptides is processed proteolytically at a single Arg residue, as well as at adjacent basic residues [23, 24].

Lamprey INS, also isolated in 1988 [39], differs from both teleostean and mammalian INSs to the same extent as the latter differ from one another. For example, lamprey INS has 14 amino acid substitutions at the variant positions when compared to porcine INS, and the same number of substitutions when compared to salmon INS. The primary structures of agnathan insulins seem to confirm that hagfishes and lampreys, have followed markedly independent routes of evolution [40]. Lamprey (*Petromyzon marinus*), as compared to hagfish (*Myxine glutinosa*), has 17 substitutions among 52 amino acids in the A- and B-chains of INS [39, 41]. These differences contrast with the known similarities in the structures of INS from related species of fish. For example, three species of Pacific salmon (*Oncorhynchus kisutch, O. keta* and *O. gorbuscha*) and three species of holocephalan fish, belonging to each of three existing families, namely, *Hydrolagus colliei, Chimaera monstrosa* and *Callorhynchus milii,* are 96–100% identical in therms of their INS structures [42–48]. Even more striking is the result, that the ray (*Torpedo marmorata*) and the shark (*Squalus acanthias*), despite the divergence in their evolution about 200 million years ago, still retain more than 90% homology in their INS structures [49, 50]. It would be worthwhile to determine whether the INS structures of various species of lamprey (or hagfish) show as much similarity within the respective groups as do the structures of salmonid or holocephalan insulins.

The amino acid sequences of several C-peptides

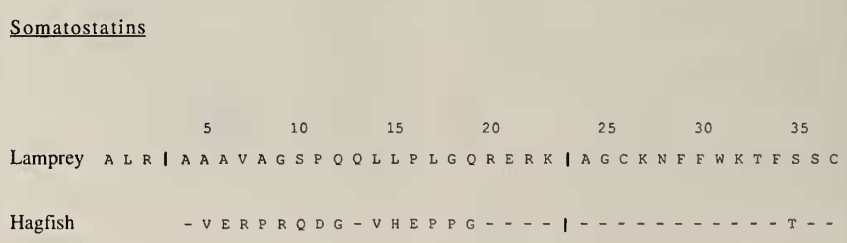

Fig. 2. Comparison of amino acid sequence of somatostatins from the lamprey (*Petromyzon marinus*) and the hagfish (*Myxine glutinosa*). From references [23 and 24]. The vertical lines indicate putative sites of processing for production of SST-14-I and SST-34-I.

from fish have been deduced from nucleotide sequences of clones of cDNA for preproinsulin [reviewed in 51]. However, only one C-peptide of fish (European eel, *Anguilla anguilla*) has actually been isolated [51]. A comparison of its structure with the predicted structures of C-peptides from hagfish, ray, anglerfish, salmon and carp has revealed that, unlike the insulins, the structural similarity among C-peptides is weak, with the exception of several amino acids in the central region of the polypeptide chain.

Information of considerable interest has appeared concerning another family of pancreatic hormones, namely glucagon (GLU) and its related peptides. This information followed the discovery of the so-called glucagon-like peptides (GLPs), the sequences of 31–34 amino acids located at the carboxyl end of the preproglucagon molecule. In contrast to the mammalian species, in which two GLPs, organized in tandem, are encoded in the same preproglucagon sequence [52], only one GLP has been isolated from teleostean fishes [anglerfish, catfish, salmon and sculpin, 3]. The same is true for the primitive holostean garfish, *Lepisosteus spatula* [53], and for a holocephalan fish, *Hydrolagus colliei* [54]. To date no GLP has been found in agnathans. However, the above mentioned studies do not exclude the possibility that piscine proglucagons may still contain more than one GLP sequence. Thus far, both GLPs (GLP-1 and GLP-2) have been found only at the evolutionary stage of amphibia: two GLPs are expressed, in the endocrine pancreas of the bullfrog, *Rana catesbeiana* just as they are in mammals [55].

Multiple, often truncated, molecular forms of SST, INS, GLU and GLP, each encoded in the same preprohormone, seem to be the rule rather than the exception in fish. Recent examples have been provided by Andrews *et al.* [23] and Conlon *et al.* [24, 54] who found multiple molecular forms of SST in the lamprey, and multiple forms of INS and GLP in the ratfish. Each of these groups of peptides probably contains the products of the same gene. By contrast, salmon may contain two preproinsulin genes that encode for two different preproinsulins, one of which is present at much higher levels [42] than the other [48, 56]. Two different insulins were discovered about thirty years ago in bonito fish [57] and quite recently in an amphibia, *Xenopus laevis* [58, 59].

The pancreatic polypeptides (PP) and their expression in fish have also been the focus of substantial attention during the recent years. Anglerfish (*Lophius americanus*) PP, named YG (glycine-extended form) or aPY, resembles neuropeptide Y (NPY) from porcine brain and the peptide YY from intestine more closely than it resembles any mammalian PP [60]. The same is true for PP from salmon [61], sculpin [22, 62] and garfish [63]. It is remarkable that such similarities seem to be confined to fish, while amphibian (bullfrog) PP is a typical bird- or mammalian-type peptide, being similar to the human PP sequence [55].

More detailed studies on anglerfish have revealed that there is more than one molecular form of NPY-like peptide in their islet organ. The majority of NPY-like peptides appear to be the YG-peptide that is expressed in a subset of islet cells, while the minor form of NPY-like peptide, closely resembling porcine or human NPY, is localized in the neurons of anglerfish brain and in islet nerves [64, 65]. It has been suggested that peptide YG is a precursor of biologically active peptide [Des^{37}-Gly]-aPY-amide [65a].

As far as we know, there are no reports of the identification of a novel peptide, pancreastatin, in fish endocrine islets. This peptide of 49 amino acids was recently purified from extracts of the porcine pancreas by Tatemoto *et al.* [66] and was demonstrated to be important in the regulation of pancreatic exocrine and endocrine secretions in mammals [67, 68].

CIRCULATING LEVELS OF PANCREATIC HORMONES AND THEIR BIOLOGICAL ACTIVITIES

The major technique used for the measurement of circulating levels of pancreatic hormones in fish has been the radioimmunoassay (RIA), although it is now evident that the enzyme-linked immunosorbent assay (ELISA) should be considered seriously as a future substitute for RIAs. It was anticipated in 1979 that non-radiometric, ELISA methods could be developed that would be as

sensitive as or even more sensitive than existing RIAs [69, 70]. Such methods are less hazardous since they do not involve the use of radiolabeled hormones and they eliminate the problem of radioactive-waste disposal.

Assays for specific measurements of pancreatic hormones in fish systems are still not common. However, assessment of INS by RIAs using piscine components are already performed in scientific laboratories in Canada, Israel, Japan, Norway, Spain, the United Kingdom, the USA and the USSR. The main obstacle for much of the potentially important fish-related research remains the lack of homologous fish hormones and antisera, although heterologous antibodies raised against insulins from scorpion fish, bonito, cod and anglerfish and their respective [^{125}I] derivatives as tracers, have been used satisfactorily in RIAs of insulins from other species of teleosts [71–78]. The need to develop more assays for teleost INS has increased since the initiation of projects directed towards the transplantation of the Brockmann bodies of fish into diabetic mammals [79] which made necessary measurements of the hormones released from the transplants.

A fully homologous RIA for fish (salmon) INS [80] has been used extensively. The results of assays of plasma INS in juvenile salmonids, in a wild population of pink salmon (*O. gorbuscha*) during their spawning migration, and in domesticated fish starved or fed specially designed diets, were reported recently [3–5, 80–86]. These results are described in more detail below.

As is now the case in mammalian studies, the regulatory effects of the novel peptides galanin and pancreastatin [66, 87] on the secretion of INS in fish and the mechanisms of their actions will probably become the focus of numerous studies as soon as these peptides are isolated from fish gut and pancreas.

Another breakthrough can be expected in the measurement of circulating levels of the second most abundant fish pancreatic hormone, a gene II SST. A fully homologous assay system for coho salmon (*O. kisutch*) SST-25-II, which has proved to be suitable for measurements in a variety of other salmonid species, was recently developed by Sheridan *et al.* [88]. Since the structure of second SST from fish, SST-14-I, is identical in fish and homeothermal vertebrates, the mammalian RIA systems should suffice for the measurement of this peptide in fish.

Unlike INS and SST-25-II, circulating levels of GLU can be assayed by mammalian RIA [77], although such assays are still rarely used. Only two groups of researchers have employed either catfish [89] or salmon [90] homologous RIA systems to measure GLU in the Brockmann body and plasma of the respective species of fish.

RIAs for mammalian glucagon-like peptide (GLP) have been described by Ørskov and Holst [91] and Oshima *et al.* [92] but we not know of any application of these RIAs to fish. It seems that antibodies raised against piscine GLP do not cross-react with mammalian GLP (Plisetskaya, unpubl.). The only published data on levels of circulating GLP in fish have been obtained by homologous salmon RIAs [85, 86, 90, 93]. Under non of the experimental conditions studied were the titres of GLU and GLP in plasma of salmonids higher than the INS titres [90]. Plasma levels of GLP were usually higher than plasma levels of GLU. The same pattern was observed after extraction of the principal islets of the fish [94, 95]. Several hypotheses have been suggested to explain the discrepancy in the yields of the two peptides that are part of the same prohormone. None has been proven. However, the differences in circulating levels of GLU and GLP have, seemingly, been provided with a logical explanation: Oshima *et al.* [92] reported that, in mammals, GLP *in vivo* is degraded more slowly than GLU. Our preliminary results from studies of incubation of isolated salmon hepatocytes in the presence of salmon GLP and GLU (Mommsen and Plisetskaya, unpubl.) suggest the same trend in fish.

While the data concerning the biological activities of INS and SST in fish continue to accumulate steadily [reviewed in 90, 96, 97], explorations into the role of GLU and, in particular, GLP in both mammals and fish made the very rapid progress during the last three years [90, 93, 96, 98–100]. The most unexpected finding was the apparently strong glycogenolytic, gluconeogenic and lipolytic effects of teleost GLP in fish [90, 101], while all attempts of find similar effects in the mammalian

liver have failed [102–105]. It was even suggested that GLP, although a member of the GLU-family, has no metabolic activity [102]. Part of the solution to this problem may have been found recently, when several research groups [106–108] reported simultaneously that the biologically active form of mammalian GLP-1 consists, not of 37 amino acids, but of 31 (sequence 7-37) which correspond to the amino acid sequence of GLP from salmon and anglerfish [94, 95].

The biological activities of GLP 7-37 (or GLP 7-36-amide) in mammals were tested primarily to determine the relation of these peptide to other pancreatic hormones, and GLP emerged as the most potent stimulator of the release of INS [106–110]. In addition GLP 7-36-amide enhances the release of SST and inhibits the release of GLU [110–111]. To reveal any insulinotropic effect of salmon GLP on perifused Brockmann bodies from fish of the same species, this peptide should be applied at concentrations at least 100-fold higher than those reported for mammals [97]. In experiments *in vivo,* the insulinotropic action of the GLP is barely detectable [90]. Teleost fishes still remain the only vertebrate group in which glycolytic and gluconeogenic fluxes are influenced by GLP [100]. By contrast, a fragment of GLU (GLU 19-29), which exhibits a potent metabolic action that is mediated by calcium ions in the mammalian liver [99, 112], seems to be without effect in piscine liver, supposedly because of the absence of analogous calcium-dependent systems [100].

Although GLPs from salmon, anglerfish and catfish all activate the production of glucose in teleosts, notable differences exist between fish species. Moreover, the actions of GLP, as is also true for GLU, are strongly dependent on the season and, probably, on the stage of the fish life cycle [100]. Both GLU and GLP seem to affect identical targets in the liver. However, the mechanisms of their action may differ: Mommsen and Moon [100] reported that no direct relationship exists between the amount of intracellular cAMP and either the metabolic action of GLU, or, in particular, of GLP. (Fig. 3). Once again, the differences between fish species are substantial [93, 101].

In this field of research more questions remain

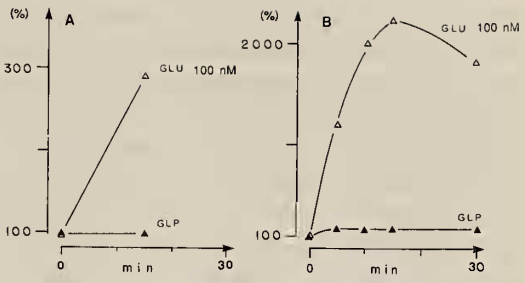

FIG. 3. Time course of intrahepatic accumulation of cAMP after application of GLU and GLP to hepatocytes from (A) trout (*O. mykiss*) and (B) eel (*Anguilla rostrata*). Values are expressed in terms of the percentage increase over vehicle-treated controls. Control levels of cAMP in salmon and eel, respectively, were 386 and 533 pmoles/g fresh weight of cells. Both bovine glucagon and salmon GLP were applied at concentrations of 10^{-7} M.
△ glucagon; ▲ glucagon-like peptide. From reference [100] (Fish. Biochem. Physiol., with permission).

to be addressed than have been answered. The first among them is: why does the metabolic action of GLP seem to be confined to fish? Does this unique property of GLP have any special physiological meaning? Can the preproglucagon be expressed in piscine organs, other than the pancreas and gut, for example, in the brain, as it is in mammals [113]?

Diurnal oscillations in piscine plasma levels of INS have been reported [83, 114] but it remains to be determined whether INS, GLU and SSTs in fish are secreted steadily or in pulses, as they are in mammals [115].

The study of the fourth group of fish pancreatic hormones, the pancreatic polypeptides (PP) has also progressed substantially during the recent years, mostly due to efforts of B. D. Noe, P. C. Andrews, A. Balasubramanian and their associates. Radioimmunoassays for anglerfish peptide YG (glycin-extended form) and for NPY-amide have been developed and aPY-like peptides in the anglerfish brain and pancreas were characterized [65, 115a]. The next logical step will probably be an attempt to assess levels of these peptides in plasma, followed by an evaluation of changes in these levels under various physiological conditions.

Anglerfish peptides belonging to the PP-family,

namely, YG and APY-NH_2, and salmon PP (NPY-NH_2) have been synthetized and tested for their biological activity in mammals. Bolus doses of natural and synthetic salmon PP and synthetic anglerfish aPY-NH_2 increased the blood pressure and decreased the heart rate in anesthetized rats in a dose-dependent manner; aPY-NH_2 diminished the volume and bicarbonate content of pancreatic juice during secretin-stimulated, exocrine pancreatic secretion in conscious dogs [116–117].

These results demonstrate that teleost PPs are not only structurally similar to mammalian NPY and PYY, but also that they can mimic NPY- and PYY-like activities in mammals [116, 117]. Of additional interest are the pilot data (Balasubramaniam, personal communication) from the direct injection of fish PP into the rat hypothalamus. This treatment enhanced the feeding behavior of the experimental animals, as does mammalian NPY [118, 119]. Consequently, we can hypothesize that PP plays a similar role in teleost fish during naturally occuring periods of either fasting or restoration of feeding. Moreover, these data appear to support the hypothesis [61] that a very low number of PP (NPY) cells and a low content of the peptide in the Brockmann body of spawning coho salmon are the correlates of a particular period in the life cycle, when the fish is naturally fasting.

In comparison to efforts in previous years, less time is now being devoted to experiments *in vitro* and *in situ* on fish Brockmann bodies and/or principal islets. Ronner and Scarpa [89], using their experimental model, which consists of a perifused isolated Brockmann body from the channel catfish (*Ictalurus punctatus*), reported a striking similarity in the responses of the catfish and higher vertebrates to hexoses. However, the catfish Brockmann body seemed to be sensitive to fewer of the common stimuli for the release of pancreatic hormones than is the mammalian pancreas.

One observation which clearly deserves more attention is that the D-cells of the catfish Brockmann body, which produce SST-14-I, are more sensitive to glucose (but not to amino acids) than INS cells [89]. This finding was confirmed by Sheridan *et al.* [88] in experimcnts *in vivo*. We can speculate that this particular pattern may be responsible for the comparatively low, and sometimes delayed, glucose-stimulated release of INS in teleost fish, as compared to the amino acid-stimulated release of INS. If, in addition, it is confirmed, in the future, that GLP in fish is not as potent a stimulator of the secretion of INS as it is in mammals [5] we will be closer to an understanding of the intolerance to carbohydrates of apparently INS-nondeficient fish.

No reports of studies *in vitro* of agnathans' pancreatic islets have appeared during the past few years.

The average plasma levels of INS, SST, GLU and GLP in the peripheral blood of teleosts are usually higher than those in mammals (cf., for example, Table 1 and 4). It is known, however, that the high levels of immunoreactive INS, observed in mammals under certain conditions, such as in cases of INS-resistant *diabetes mellitus*, are in fact caused by an increase in levels of proinsulin in the immunoreactive pool, measured as INS [120]. Conlon and Thim [51] recently isolated the first teleostean (eel) C-peptide of proinsulin. It is to be hoped that this accomplishment will provide researchers with a tool for the assessment of levels of proinsulin in fish, so that the question of the contribution by proinsulin to plasma levels of INS in fish and to binding to receptors can finally be addressed.

Our knowledge of plasma levels of pancreatic hormones in fish has resulted in a clear change in the attitudes of comparative physiologists and biochemists: most of them are turning now to usage of "physiological doses" of hormones for experiments both *in vivo* and *in vitro*. However, the question remains as to whether the levels of hormones that are routinely measured in the peripheral blood correspond to those that the liver cells actually "see".

As in mammals, the fish liver is the major organ for glucose homeostasis, the primary target for pancreatic peptides and an important site for their degradation [121]. Consequently, it is logical to expect that in fish, as in mammals, the liver is exposed, through the hepatic portal vein, to much higher levels of pancreatic peptides than is any other organ. However, untill 1989 no actual values for levels of pancreatic peptides circulating in

Table 1. Titres of some pancreatic hormones in the peripheral blood of various salmonids

Species	Insulin (ng/ml)*	Glucagon	GLP	SST-25-II	References
O. kisutch	0.9–15.0	0.01–2.00	0.10–2.30	0.15–1.20	*INS, GLU, GLP*
O. tshawytscha	1.5–9.1	0.05–0.06	0.20–2.80	0.15–1.20	[3, 4, 80, 83]
O. gorbuscha	0.2–3.0				[85, 86, 90, 17]
(wild population)					*SST-25-II*
O. mykiss	1.7–48.0	0.01–2.00	0.10–2.10	0.15–1.20	[88]
S. salar	2.0–40.0	0.01–1.60	0.05–1.90		
*S. gairdneri***	0.1–4.2				
(steelhead, wild population)					

* The values represent a summary of results of many different assays of fish of a particular species (60–200 fish per group), but of different age, sex and feeding conditions, maintained on various diets and at various water temperatures. For variations in levels of hormones in uniform groups of fish see the original publications.
** New species name for *Salmo gairdneri* is *Oncorhynchus mykiss*.

various blood vessels of the same fish could be found in the literature. Such values were obtained from an adult trout and published recently [122]. As could have been expected, levels of INS, GLU and GLP in peripheral blood constituted only 20-30% of those in the hepatic portal vein (Table 1). There is no doubt that differential blood sampling from various vessels, if continued in studies on fish, will provide us with new insights into the uptake and processing of biologically active peptides by their target tissues.

Precise information about the concentrations of pancreatic hormones at the "entrance" to and "exit" from the liver will be of benefit to several groups of researchers who are experimenting with slices of fish liver or isolated hepatocytes [93, 100, 121, 123]. These studies have become even more challenging since the publication of the new concepts that have been introduced into investigations on mammalian liver. The idea [124] that the liver acinus is a unit of hepatic microcirculation seems to be generally accepted by mammalian physiologists [125]. Three distinct metabolic zones in liver acini, namely, the periportal, perivenous (or pericentral) and intermediate zones have been proposed. Morphological, histochemical and biochemical differences between these zones [125, 126] and some differences in the hormonal regulation of gluconeogenesis and ketogenesis in periportal and perivenous rat hepatocytes have been reported [127]. Methods have been developed for the separation of so-called periportal and perivenous liver cells from mammals [128–131]. Whether this idea of metabolic zonation can be applied to the piscine liver, microstructure of which differs in many ways from that of the mammalian liver [132–134], remains controversial. The first studies on two populations of hepatocytes separated from the same liver of trout and catfish by pulses of digitonin that were followed by digestion with collagenase and Percoll-gradient centrifugation, were undertaken by Mommsen *et al.* [135] and Ottolenghi *et al.* [136]. Although some metabolic differences between "periportal" and "perivenous" pols of cells were found, both studies failed to reveal either a real "metabolic zonation" or different responsiveness to glucagon, in terms of carbohydrate fluxes, in these cells. Improved methods for the separation of cells may be needed for future research in this field.

HORMONE-RECEPTOR INTERACTIONS

In sharp contrast to extensive investigations in mammalian systems the receptors for pancreatic hormones in fish tissues remain almost completely unexplored [2], although the cyclostome and fish hormones, in particular INS, have been tested for their binding to mammalian plasma membranes. These studies have concentrated mostly on evalua-

tions of the potency of newly isolated piscine hormones, as compared to their mammalian counterparts. Fish pancreatic peptides either do not bind to mammalian receptors or have much lower binding affinities than do the mammalian peptides [42, 54, 105, 137–140]. The same pattern (lower binding affinity as compared to mammalian INS) has been found when agnathan or piscine insulins are bound to the receptors in homologous tissues [141–144]. Report on the structure of the INS receptor in the plasma membranes of the hagfish (*Myxine glutinosa*) liver [145] supports an earlier conclusion that the receptor protein has been much better conserved in the course of vertebrate evolution than the ligand [141, 142, 146]. The partial amino acid sequence of the INS receptor from coho salmon (S. Chan, personal communication) lends further support to this hypothesis. However, an INS receptor from the stingray (*Dasyathis americana*) liver [147] seems to display some peculiar features in its structure. It has been reported to be a dimer, consisting of two identical subunits, each with a binding (alpha) and a tyrosine kinase (beta) domain. Stuart [147] suggested that the stingray receptor is not completely cleaved. This stands in marked contrast to all other (mammalian, avian, reptilian and amphibian) receptors for INS, which are tetramers, built from two extracellular alpha subunits and two transmembrane/intracellular beta subunits. The alpha subunits are connected to one another and to the beta subunits by disulfide bonds [148–151]. The same description seems to be applicable to the INS receptor from *Drosophila melanogaster* [152] which has a subunit structure similar to that of the mammalian INS receptor. By contrast, the proposed structure of the stingray INS receptor [147] more closely resembles that of receptors for IGF II and for EGF.

Competitive binding of INS and IGF to the INS receptor of the stingray led Stuart [147] to the conclusion that, at the phylogenetic stage of the elasmobranch fish, both INS and IGF may transduce their signals through the same receptor. Some overlap in structure, specificity and function between receptors for IGF-I, IGF-II and INS has been reported recently in the early chick embryo and in the lizard brain [151, 153]. Nevertheless, recent studies of Gutiérrez and Plisetskaya [154] on liver plasma membranes from salmon demonstrated binding with much higher affinity for INS than for either mammalian IGF-I or IGF-II. Similar studies should be undertaken with a wider range of lower vertebrates and lower chordates. Such investigations appear especially relevant since the report by Chan *et al.* [155] that the overall organization of the preproinsulin gene of an amphioxus (*Branchiostoma lanceolatum*) indicates close relation to both INS and IGF-I.

It is widely accepted, that, in mammalian INS-sensitive tissues, the numbers of receptors for INS on the plasma membrane are regulated by the circulating levels of INS [156–158], so that elevation in levels of INS causes a decline in the number of receptors. This assumption has been extrapolated to the properties of the INS receptors in fish. Therefore, when Ablett *et al.* [159] found that isolated hepatocyte from trout reared on a high-carbohydrate diet had more specific binding sites for INS than those from control fish, it was concluded that the plasma INS levels in this fish were low. Developing this idea still further, Christiansen and Klungsøyr [160] suggested that, because of the strictly reciprocal relationship between the concentration of receptors and plasma levels of INS, the assessment of receptor binding can provide an estimate of INS levels in the course of the nutritional studies on fish. Since the high-carbohydrate diet resulted in an increase of the numbers of specific binding sites for INS in trout liver [159], it was concluded that glucose does not stimulate the secretion of INS in the rainbow trout.

Such a conclusion from the abovementioned experiment is difficult to accept. First of all, glucose does indeed stimulate the secretion of INS in agnathans and in teleost fishes [71, 143, 161], including the rainbow trout [81], though it is not as potent in this respect as amino acids. Secondly, the question should be addressed as to whether the reciprocity between plasma levels of INS and number of receptors, as reported in mammals, should be applied to fish. Two recent publications [162, 163] provide some evidence that, at least in the Baltic lamprey (*Lampetra fluviatilis*), neither natural INS deficiency, caused by prolonged prespawning anorexia, nor hyperinsulinemia induced

by injection of insulin, changes the binding parameters of INS in the liver, heart muscle or brain. Moreover, the data obtained from mammals [164] indicate that, at least, receptors for INS from brains of adult animals are unaffected by alterations in the levels of circulating INS. In primary cultures of cortical cells from fetal mice, elevated concentrations of INS in the media cause "up-regulation" of INS receptors [165].

Leibush [144] commented that since the "down-regulation" phenomenon is related, most probably, to the internalization of the receptors for INS, a process that does not take place at low temperatures, this phenomenon could hardly be expected to occur in either agnathans or fish that are maintained at low water temperatures. It is likely that the problem is even more complicated, since Gutierrez *et al.* [166] and Gutierrez and Plisetskaya [154] found either the presence or the absence of down-regulation, dependent not only on environmental temperature but on some unidentified physiological conditions of fish. Whatever may be the mechanism that governs the number of the receptors for INS in fish, it is clear that the time has come to examine it in more detail. The same is true for other pancreatic peptides, such as GLU and GLP. While the receptors for GLU and the transduction of signals from the cell surface to the intracellular targets have been analyzed in great detail in mammals [99, 112, 167, 168] no similar studies have been undertaken in fish. However, some indirect information about receptors and post-receptor events is available. Clear differences are already apparent at the level of binding of GLU to its receptor. For example, catfish glucagon, although very close in terms of structure to mammalian GLU, does not bind to GLU receptors in the liver, pituitary or hypothalamus of the rat. It does not activate adenylate cyclase in any of these tissues, while its porcine counterpart strongly activates this enzyme. By contrast, catfish GLP does stimulate the activity of hypothalamic and pituitary adenylate cyclase in rats [105]. Postreceptor transduction of signals also seems to differ between fish and mammals, especially in regard to the stimulation of adenylyl cyclase activity, which can be achieved only after treatment with pharmacological concentrations of GLU (see above and Fig. 3).

As far as we know, there is at present no available information about hormone-receptor interaction of peptides that belong to the gene II SST- or to PP families in fish.

PANCREATIC HORMONES IN FISH UNDER VARIOUS PHYSIOLOGICAL CONDITIONS

a) *Smoltification*

Smoltification or the parr-to smolt-transformation in salmonids involves profound morphological and physiological changes. This crucial period prepares juvenile salmon for downstream migration as well as for survival at sea. Smoltification includes changes, mostly increases, in the titres of many hormones, such as thyroxin, GH, prolactin, catecholamines, sex steroids, cortisol and some others [169–171]. It is surprising that pancreatic hormones have received only scant attention in this regard [169]. The first pancreatic hormone measured during the entire period of the parr-to-smolt transformation was INS [83]. In experiments continued from 1984 to 1989, the plasma profiles of this hormone revealed an annual peak at a specific time, namely at the very beginning of the transformation of parr to the transitional stage (Table 3). The annual peak was followed by a rapid or gradual decline in levels of INS. It is noteworthy

TABLE 2. Circulating levels of insulin in plasma of juvenile coho salmon, *Oncorhynchus kisutch,* in 1985; According to Plisetskaya *et al.* [83]

Date	Insulin (ng/ml)	Stage
February 1	2.0±0.2 (9)*	Parr
February 15	1.1±0.1 (9)	Parr
March 15	1.0±0.1 (8)	Parr
March 29	7.7±1.7 (6)	Parr-transitional
April 6	1.9±0.4 (7)	Parr-transitional
April 12	0.8±0.1 (7)	Transitional-smolt
May 3	0.8±0.1 (8)	Smolt
May 17	0.6±0.1 (7)	Smolt
May 24	1.1±0.2 (7)	Smolt
June 21	2.4±1.0 (8)	Smolt transferred to seawater

* In parentheses: number of samples.

Table 3. Circulating levels of glucagon and glucagon-like peptide in plasma of juvenile coho salmon, *Oncorhynchus kisutch,* in 1989

Date	Glucagon (ng/ml)	Glucagon-like peptide (ng/ml)	Stage
February 22	0.40±0.08 (4)*	1.60±0.20 (4)	Parr
March 8	1.00±0.04 (4)	1.10±0.04 (4)	Parr
March 21	0.50±0.06 (7)	1.00±0.10 (7)	Parr-transitional**
April 3	0.60±0.06 (8)	1.70±0.26 (8)	Parr-transitional
April 17	0.50±0.02 (5)	0.80±0.17 (5)	Smolt
April 26	0.70±0.06 (4)	2.00±0.32 (4)	Smolt
May 5	2.30±0.40 (6)	1.10±0.18 (6)	Smolt

* Each sample of plasma represents a pool from 2–3 fish.
** Insulin levels rise to a maximum of 7–10 ng/ml; data for insulin are not shown but the profiles are similar to those presented in Table 2.

Table 4. Titres of pancreatic hormones (ng/ml±S.E.M.) in salmonids that were either fed or fasted

Hormone	Fed	Fasted	Species	Details	Reference[2]
Insulin	6.8±0.60 (10)[1]	1.6 ±0.30 (10)	*S. gairdneri*	Fasted 1 week	[73*]
	4.2±0.30 (10)	2.2 ±0.30 (10)		Fasted 1 week	
	4.5±0.80 (10)	1.4 ±0.20 (10)	*O. kisutch*	Fasted 1 week	[80**]
	4.3±0.80 (9)	0.9 ±0.10 (9)		Fasted 2 weeks	
	12.1±1.10 (10)	2.0 ±0.10 (10)	*O. mykiss 1986*	Fasted 6 weeks	[85**]
	13.0±1.70 (15)	2.2 ±0.10 (15)	*O. mykiss 1988*	Fasted 6 weeks	[86**]
	10.9±0.70 (30)	3.0 ±0.10 (26)	*O. mykiss 1989*	Fasted 6 weeks	[191**]
Glucagon	0.80±0.10 (10)	0.20±0.10 (10)	*O. mykiss*	Fasted 6 weeks	[85**]
	0.12±0.05 (15)	0.08±0.03 (15)	*O. mykiss*	Fasted 6 weeks	[86**]
	1.60±0.10 (29)	1.20±0.20 (25)	*O. mykiss*	Fasted 6 weeks	Plisetskaya, (unpublished)**
GLP	0.60±0.10 (10)	0.30±0.04 (10)	*O. mykiss*	Fasted 6 weeks	[85**]
	1.10±0.04 (10)	0.80±0.01 (10)	*O. mykiss*	Fasted 6 weeks	[86**]
	1.90±0.30 (28)	0.40±0.02 (25)	*O. mykiss*	Fasted 6 weeks	Plisetskaya (unpublished)**

[1] In parentheses: numbers of fish assayed. [2] Radioimmunoassay with * cod components; ** coho salmon components

that neither the maximum number of the receptors for INS in the liver nor the maximum binding of INS to the liver plasma membrane, were coincident with this surge in levels of INS [154]. The titres of members of the glucagon family peptides, namely GLU and GLP, assessed during smoltification in 1989, fluctuated without any particular periods of significant elevation or decline (Table 4).

The surge of levels of INS seems to be consistent with a switch in metabolic conditions, as the clearly anabolic parrs become catabolic smolts [172, 173]. Changes in the secretion of INS evidently play a major role in these metabolic shifts, as was demonstrated in model experiments that included either inactivation of INS in parr by injection of INS-specific anti-serum or administration of INS to smolts. After these experimental treatments, parrs acquired some metabolic features of smolts and *vice versa.*

Within several weeks after entering the sea water, smolts of Pacific salmon usually regain high plasma levels of INS which are favourable for a rebuilding of stores of lipid and glycogen. In

juvenile fish that reach the sea prematurely and cease their normal growth ("stunting" phenomenon), levels of INS remain low [83, 169].

b) *Spawning migration*

Profiles of plasma INS were assessed in wild population of Pacific salmon (*O. gorbuscha*) along their way to the spawning grounds [82, 174] and in reproductively maturing Atlantic salmon, *S. salar* [175]. Fish of both species, although anorexic during the spawning period, maintain relatively high levels of plasma INS. Metabolic studies on the liver cells isolated from anorexic *O. keta,* at four sampling sites along their 1150-km migration route, led French *et al.* [176] to the conclusion that spawning of sockeye salmon is apparently supported by the catabolism of carbohydrates. The final depletion of carbohydrate reserves from both muscles and liver of Pacific salmon occurs during or immediately after spawning. In maturing salmon, INS may participate in the preservation of these carbohydrate reserves, accumulated mostly *via* gluconeogenesis, from premature exhaustion. It is remarkable, that relatively high levels of INS during spawning migration in anorexic fish coincide with enhanced gluconeogenesis while, in normally feeding fish, INS is believed to act to reduce gluconeogenesis [177]. Therefore, the interaction between INS and gluconeogenic fluxes in anorexic fish should be reevaluated and studied in greater detail.

In Atlantic salmon, the elevation in levels of INS, GLU and GLP is observed in spring prior to the onset of maturation, when the fish continue to feed extensively [175, 178]. Similar changes have previously been described for the scorpion fish, *Scorpaena porcus* [161], and for the sea bass, *Dicentrarchus labrax* [179]. Although some involvement of INS in the regulation of the uptake of vitellogenin has been reported [180] and binding sites for INS have been found on the plasma membrane of the ovaries of *S. salar* (Gutiérrez, personal communication), details of the role of INS in the maturation process await further investigation. Another possibility that deserves to be explored is the direct transfer of INS from the blood of females into the eggs prior to spawning.

The spring elevation in plasma levles of INS in Atlantic salmon seems to coincide with an increase in levels of GLU and GLP, an observation that again contradicts the relationship between these hormones in mammals. The surge in levels of INS in the spring may enable the maturing fish to increase their stores of protein, lipids and carbohydrates in somatic tissues, in anticipation of maturation. It is possible that, when these metabolic stores reach some particular level, they may signal the readiness of fish for sexual maturation and for the switch from somatic to gonadal growth. After feeding has ceased, the gonads, and in particular the ovaries, continue to incorporate proteins and lipids stored in the somatic tissues [175].

In both Pacific and Atlantic salmon at the time of spawning, males have higher plasma levles of INS than do females [82, 178]. This difference may be caused by the necessity of meeting an increased demand for energy, since the males arrive at the spawning grounds before the females so that they can defend their territory, and the males remain there for a longer time to engage in multiple spawnings [175]. An alternative explanation for the difference in INS levels is that, in females, some INS in transferred from the circulation to the eggs.

Is the hormonal regulation of metabolism in fasting fish similar (albeit slow because of their natural heterothermic conditions) to the regulation of metabolic fluxes in other vertebrates? Such a comparison is difficult to make because the fish that have been studied are mostly carnivorous, while common laboratory mammals and birds are omnivorous. Fortunately, in this regard, a group of French scientists has conducted a thorough study of the physiology and biochemistry of the long-term, natural fasting in penguins. Their study included measurements of metabolic indices and the regulation of these indices by pancreatic hormones [181–183]. The experimental subjects, chicks of the king penguin (*Aptenodytes patagonica*), experience a natural 4-to-6 month fast during the subantarctic winter. These birds are strictly carnivorous. Therefore, the results accumulated by the French group should be suitable for comparisons with the data obtained from fish. The researches distinguish two phases of the natural

fast: the first is accompanied by the early metabolic adjustments that are characterized by a decline in the plasma levels of hormones (including INS) and a decline in the utilization of energy sources. During the next, long-term phase of the fast, the levels of INS and GLU remain stable [182, 183], a situation that resemles conditions in migrating fish.

c) *Experimental fasting* versus *feeding in teleost fishes*

Because of their remarkable ability to tolerate long non-feeding periods, fish are becoming valuable experimental models for studies of the metabolic patterns of fasting conditions. The metabolic strategies of previously actively feeding fish, which are then experimentally deprived of food, seem to differ from those described above for upstream-migrating anorexic fish. At the same time they bear some similarity to the early period of fasting in penguins [182].

The elevation of plasma levels of GLU observed in the sea bass by Gutierrez *et al.* [184] and in the juvenile Atlantic salmon by Sundby (personal communication), is not easily detectable. Even if such an elevation occurs it lasts for only a short period of time (usually during fourth and fifth days after withdrawal of food). Moreover, in contrast with the situation in mammals [85], in fish plasma the molar ratios of GLU to INS never reach, not to mention never exceed, 1.0, with levels of GLU always remaining lower than those of INS. After this initial period, experimental fasting leads to a decline in plasma levels of INS, GLU and GLP (Table 4). This decline is, however, not uniform: levels of INS drop more rapidly than levels of GLU and GLP. As a result, the molar ratios of GLU and, especially, of GLP to INS tend to increase during the course of starvation, and this increase favours an enhancement of the activities of gluconeogenic enzymes and, consequently, the gluconeogenic potential of the liver [85, 86]. At the same time, as has been demonstrated in experiments on chinook salmon *in vitro*, fasting for three weeks sharply diminishes the responsiveness of the liver slices to the glycolytic action of GLU [185].

We have not discussed the role of hormones in fish growth in this survey. The subject was well presented in both 1986 and 1987 [186, 187]. Deciphering of the structure of the first identified piscine IGF [188], as well as the findings that the expression of IGF in piscine liver is regulated by the GH [188] and that an injection of GH elevates levels of immunoreactive IGF in fish plasma [189] open new and fertile areas for exploration of the mutual interrelationships between GH, IGF and INS in the regulation of fish growth and metabolism. There in no doubt that stunted fish, which possess high levels of plasma GH [187, 190], coincidental with low levels of INS [83], may be considered an ideal model for these studies.

CONCLUSION

The divergent life cycles and feeding habits of fish, their consistent growth, and amazing tolerance of long periods of food deprivation are characteristics that keep fishes in a central position in studies of the adaptive evolution of metabolism and its regulatory mechanisms in vertebrates.

Pancreatic hormones play the pivotal role in these regulatory mechanisms, and an overview of the recent literature demonstrates the growing importance of fish as an experimental model for studies of the structure and functions of these hormones. There is no doubt that, if the pace of studies of pancreatic hormones in fish remains the same as it has been during the past several years, answers to the majority of the questions addressed in this review will soon become available.

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